MR400 Operating Procedures

1. Log in as an operator by selecting your group from the drop-down list and entering your group's password.

2. Insert your sample (NMR tube must be at least 8" long) in an Agilent spinner and adjust the sample's position in the spinner with the Agilent sample depth gauge. Never use Bruker spinners or depth gauge on the Agilent MR400 or vice versa.

3. Pick an available (gray, green, or red) and optimal position for your sample:

    a. If the instrument is idle, put your sample one up from the center position, *e.g.*, if the center position is 5, put yours in 6. Do not put your sample in the center position.

    b. If there are samples running and waiting, put your sample after the last one in the queue. Note that the sample tray rotates clockwise, and that samples should be arranged counter-clockwise in the tray and one after another, *i.e.*, do not skip positions. The sample changer is known to misbehave when positions are skipped.

4. Climb up the ladder so that you are able to reach both the front and the back of the tray with ease, put your sample in your chosen position by holding the top of the NMR tube (not the cap!) and lowering it down slowly until the tray catches the bottom of the spinner before letting go of your sample. Do not touch the magnet.

5. Click on [New Study], then

    a. Check and make sure there is enough time left for your sample (see <12.g> below)

    b. Select DayQ or NightQ (default is DayQ, this must be set before selecting experiments)

    c. Select experiments to run from the Experiment Selector panel on the upper left

    d. Enter a sample name in the *Sample Name* field (required)

    e. Enter your name in the *Notebook* field (required) – Your data will be saved in this subdirectory. Be consistent with what you enter here. Note that it's case sensitive.

    f. Select a solvent (lock, shim and tune should be left at default)

6. Optimize acquisition parameters. Double click on an experiment and change the parameters to suit your needs (see <12.h> for parameter setting recommendations), then click on the [Save] button above the parameter panel, which will update the parameters, the time for the experiment, and the total time for the sample.

7. Select your sample's position on the Tray Panel, making sure it matches what you have selected in step 3 above.

8. Click on [Submit]

9. If you have another sample to set up, repeat step 2 - 8. Otherwise, you are done.

10. When done, click on the [Logout] button on the opposite side of the [Start] button. Do not log out of the walkup Linux account!!!

11. Sign the logbook – PRINT your name (not just your initials) and fill in the rest of the required information. You are required to sign the logbook every time your use the instrument.

12. *Additional Information:*

a. Due to the depth of the sample tray, only 8" or longer NMR tubes should be used on this instrument. To keep things simple, I recommend phasing out 7" tubes and order only 8" tubes in the future.

b. Shigemi tubes for Agilent/Varian instruments cannot be used on Bruker instruments due to the differences in the lengths of the bottom filling, which is 15mm for Agilent/Varian and 8mm for Bruker. However, Shigemi tubes for Bruker should be usable on Agilent/Varian instruments.

c. Bruker and Agilent/Varian spinners are not interchangeable. Never use a Bruker spinner on an Agilent/Varian instrument or vice versa.

d. Bruker and Agilent/Varian sample depth gauges are not interchangeable. Never position a sample to be run on a Bruker instrument using an Agilent/Varian depth gauge – damage to the probe will occur if you do that.

e. Sample position color coding: **blue** – in progress (in **dark blue** when sample is in pre-acquisition, e.g., tuning, shimming, etc); **green** – finished; **red** – error; **yellow** - day queue, **purple** - night queue, and **gray** - open.

f. When all experiments are finished for the sample, the sample will be ejected and return to its original position. Do not remove the sample before it settles down in the tray. Do not remove or place samples in the tray while the instrument is changing samples and the tray is moving.

g. There are two ways to find out when your sample will be run and/or how much time is left in the NightQ: 1) In the lower right under Current Status after clicking on [New Study]. It will show the current submission start time for both DayQ and NightQ. 2) In the lower left corner under View: Spectrometer, scroll all the way down and mouse over "Studies in day queue" or "Studies in night queue" and the total queue time will be shown, which is not as accurate because does not include the currently running sample.

h. List of common experiments and their recommended parameter settings. Note that all 2D experiments are set up to acquire with non-uniform sampling which will cut the experiment time by half.

- ➤ **H1** – Spectral window (default -2 to 14ppm), nt (default 16, 1 minute)
- ➤ **C13** – Spectral window (default -10 to 230ppm), nt (default 256, 10 minutes; need 512 scans for a 10mg sample with MW < 500)
- ➤ **F19** – Spectral window (default -204 to 34ppm), nt (default 32, 1 minute; need about 25% more scans than 1H)
- ➤ **P31** – Spectral window (default -50 to 200ppm), nt (default 64, 2 minute; it's about 380 times more sensitive than 13C)
- ➤ **B11** – Spectral window (default -200 to 200ppm), nt (default 16, should be 128 instead; it's about 150 times more sensitive than 13C, but with much broader linewidths and a prominent background hump from the NMR tube material)
- ➤ **gCOSY** – nt (≥2, depends on concentration and size of coupling constants), ni (200 – 400, depends on spectral width and degree of overlapping), spectral window (inherited from the required 1D 1H and should be optimized on that 1D 1H). Default nt=2, ni=200,

6 minutes.

- ➢ **gHSQCAD_PS** – nt (≥2, depends on concentration), ni (200 – 400, depends on spectral width and degree of 13C signal overlapping), 1H spectral window (inherited from the required 1D 1H and should be optimized on that 1D 1H), 13C spectral window (should be optimized to include only 13C's with directly attached 1H). Default nt=2, ni=200, -10 to 190ppm 13C window (remember to optimize!), 10 minutes.

- ➢ **gHMBCAD** – nt (at least 4 to 6 times the nt for HSQC), ni (256 – 512, depends on 13C spectral width and degree of overlapping), 1H spectral window (inherited from the required 1D 1H and should be optimized on that 1D 1H), 13C spectral window (should include ALL 13C's). Default nt=16, ni=256, -15 to 225ppm 13C window (remember to optimize!), 1 hour 25 minutes.

- ➢ **NOESY** – Whenever possible, choose the 1D selective version of this experiment (NOESY1D) over this, with same parameter settings. nt (10 to 15 times the nt of a normal 1H, depends on concentration and size of NOE), ni (200 – 500, depends on spectral width, degree of overlapping, and 1H – 1H spacial distance or size of NOE), NOE mixing time mixN (0.5 to 1sec for small molecules), spectral window (inherited from the required 1D 1H and should be optimized on that 1D 1H). Default nt=16, ni=256, mixN=0.6, 2 hours.

- ➢ **ROESYAD** – Used for medium-sized molecules (MW 800 – 1000) where NOE might be zero. Whenever possible, choose the 1D selective version of this experiment (ROESY1D,) over this, with same parameter settings. nt (10 to 15 times the nt of a normal 1H, depends on concentration and size of ROE), ni (200 – 500, depends on spectral width, degree of overlapping, and 1H – 1H spacial distance or size of ROE), spinlock mixing time mixR (default 0.2, could be up to 0.5 for weaker ROE interactions), spectral window (inherited from the required 1D 1H and should be optimized on that 1D 1H). Default nt=16, ni=256, mixR=0.2, 2 hours 15 minutes.

- ➢ **zTOCSY** – nt (similar to gCOSY), ni (200 – 400, depends on spectral width and degree of overlapping), spinlock duration mixT (0.02 to 0.12. 0.02 will show cross peaks mainly from geminal and vicinal protons similar to gCOSY, longer mixing time will show cross peaks from further away protons in the same spin system), spectral window (inherited from the required 1D 1H and should be optimized on that 1D 1H). Default nt=2, ni=200, mixT=0.08 (show cross peaks from protons up to 5 or 6 bonds away), 9 minutes.

i. Delete a sample before it starts: You can delete a sample in the queue before it starts, *i.e.*, either yellow or purple, by right-clicking on the sample number on the Tray Panel and select [Delete Study]. Do not delete an active sample! An active sample can not be deleted and should be stopped instead.

j. Modify a sample before it finishes: You can modify a study in the queue or in progress (such as adding, deleting, or modifying an experiment) by right-clicking on the sample number on the Tray Panel and select [Edit Study]. Note that if the study is in progress, only experiments that have not started can be deleted or modified. When done modifying, click on [Submit] to confirm the change or click on [Done] to cancel the change.

k. Stop an experiment after the position turns light blue: Do not try to stop an experiment while the position is in dark blue! A running experiment can be stopped via {Automation} menu -> {Automation Controls} -> {Stop-save-resume} (if you want to save the data up to

this point) or {Stop-discard-resume} (if you don't want to save the data). This will stop the current experiment without error and move on to the next experiment or sample in the queue.

l. Do not log out of the walkup Linux account!

m. Troubleshooting: If the sample tray fails to move or moves to the wrong position, enter on the VnmrJ command line resetrobot and wait for the tray to stop at position #1. Then resume the automation queue by selecting {Automation} -> {Automation Controls} -> {Resume Automation}.

n. Schedule and Rules:

Weekdays:
7:00AM – 9:00AM	DayQ, unlimited. Submission starts at 7:00AM
9:00AM – 6:00PM	DayQ, 30 minutes per user (see the one hour rule below)
6:00PM – 7:00AM	NightQ, unlimited. Submission starts at 4:00PM

Weekends:
9:00AM – 5:00PM(Sat.)	DayQ, 60 minutes per user (same one hour rule applies)
5:00PM(Sat.) – 7:00AM(Mon.)	NightQ, unlimited. Submission starts at 1:00PM on Saturday

- The one hour rule: One hour *after the finish* of your previous sample(s), you may submit another 30 minutes of samples. There is no limit on how many 30 minutes blocks one can have in a day as long as the one hour rule is followed.

- You must check and make sure there is enough time left in the DayQ or NightQ for your experiments before submitting your samples (see <12.g> above).

- The total number of NightQ samples submitted by all users before 6pm on weekdays or 5:00PM on Saturdays should be no more than 6, so that DayQ users will have at least 6 sample positions available.

- Please observe the NightQ submission start time. I intend to check, and early submissions will be deleted without notice.

- Use of this instrument is on a first-come, first-served basis. No reservation is required or allowed. If you have a need that cannot be met because of the above policy, please let me know so that alternative arrangement can be made and/or the policy can be adjusted.